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ANTIBODY AS DEMONSTRATED BY COUNTERIMMUNOELECTROPHORESIS

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IMMUNOLOGICAL CHARACTERISTICS OF INFLUENZA PRECIPITATING ANTIBODY  
AS DEMONSTRATED BY COUNTERIMMUNOELECTROPHORESIS \*

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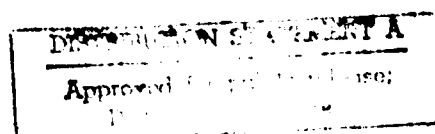
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## ABSTRACT

Antibody to influenza infection was determined by counter-immunoelectrophoresis (CIE). The antibody resulting from influenza disease reacted to both related and distant strains of influenza A antigen but not to influenza B antigen. CIE antibody was not demonstrable following immunizations with inactivated influenza vaccine. While influenza antibody, as demonstrated by CIE, was always associated with elevated complement fixation antibody, the reverse was not the case. Since the precipitating antibody was only detected in those individuals with influenza disease, the test could serve as an early alert signal during surveillance of an impending influenza A epidemic.

## INTRODUCTION

Counterimmunoelectrophoresis (CIE) has been used for the rapid detection of antigens (2, 3, 4, 5) and to a limited extent antibodies in human sera (1, 6, 7). The report by Berlin and Pirojboot (1) that a precipitating antibody to influenza A developed rapidly after overt disease onset suggested that the method might be applicable to surveillance for influenza A antibody. The detection of precipitin antibody would serve as an early alert signal that influenza may become a major epidemic.

The purpose of this communication is to compare the characteristics of the influenza precipitating antibody as demonstrated by the CIE technique with the complement fixation (CF) antibody contained in sera from human subjects infected with influenza or from vaccinated persons.

## MATERIALS AND METHODS

The CIE technique and the preparation of the influenza A antigen was as previously described (1). A/Hong Kong/1/68 ( $H_3N_2$ ) antigen was derived from infected Rhesus monkey kidney tissue cells (MK). Other antigens such as A/PR/8/34 ( $H_1N_1$ ), A/Ann Arbor/1/57 ( $H_1N_1$ ), A/Japan/170/62 ( $H_2N_2$ ), A/England/42/72 ( $H_3N_2$ ), and B/MASS/5/72 were similarly prepared for strain specificity studies. All antigens had a CF titer of at least 1:16 before use in the CIE test. CF antibody to influenza types A and B were measured by microtechnique procedures (8).

Serum donor sources and sampling. (i) Non-vaccinated subjects.

(a) Asymptomatic individuals (Study group 1a):

Twenty-seven Navy recruits who developed CF antibody seroconversions, (4 fold or greater titer increase) to influenza A during their nine week training period. Serial serum specimen on each individual had been collected at beginning, mid-point (5th week) and at the end of training (9th week).

(b) Influenza patients (Study group 1b and 1c):

Eight recruits hospitalized for confirmed influenza A infections by virtue of virus isolation and/or a stable elevated ( $\geq 1:64$ ) CF titer (group 1b) or seroconversions between paired serum specimens (group 1c) obtained at onset of hospitalization and three weeks later during convalescence.

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Four laboratory workers with influenza disease donated either acute and/or convalescent phase serum specimens. Sera was tested for CIE precipitin responses to  $H_1N_1$ ,  $H_1N_1$ ,  $H_2N_2$ , and  $H_3N_2$  strains of influenza A CF antigens and B/MASS CF antigen.

Three recruits with influenza symptoms who yielded influenza B isolates and CF seroconversion to B/MASS/5/72 antigen.

(ii) Vaccinated subjects.

- (a) Twenty asymptomatic recruits donated serum specimens prior to and three weeks post vaccination with a bivalent preparation containing 400 CCA units each of influenza A ( $H_3N_2$ ) and B antigens. All subjects seroconverted by CF (four fold or greater).

All of the above sera were tested for CIE precipitating antibody to influenza type A antigens and simultaneously retitered for CF antibody. In addition, four sera which demonstrated both precipitin and elevated CF antibody titers were fractionated on Sephadex G200 using PBS (0.01M Phosphate, 0.15M NaCl, pH 7.0) as eluent. Fractions were tested for IgM and IgG immunoglobulins (Quantiplate, Kallestad Inc., Chaska, MN) and titers for CF and CIE on the various fractions were determined as described.

## RESULTS

The results in Fig. 1 compare the mean influenza A CF antibody titers and presence/absence of CIE precipitating antibody of the three study groups. The data in study group 1a shows the temporal relationship of the CF antibody of 27 subjects at various times during training (0, 5, 9 weeks) with the CIE precipitating antibody. All 27 subjects were negative for CIE precipitating antibody at the 0 and 9 week sampling while all 27 gave a positive precipitin reaction at the 5th week sample. It is apparent that while the CF antibody titer of the 27 subjects was detectable in the 5th week samples, and remained elevated at the 9th week of training, the CIE precipitating antibody was no longer detectable in any of the 9th week serum samples. It appears, however, a positive CIE reaction can be demonstrated for at least 3 weeks (Fig. 1c). CIE precipitating antibody is not demonstrable in subjects without overt disease (Fig. 1a) or early in the acute phase of the disease (Fig. 1c).

The CF and CIE antibody activity from immune globulin fractions is shown in Fig. 2 and Table 1. Although both the CF and precipitating antibody was eluted with the IgG class of immunoglobulins, they did not demonstrate identical reactivity profiles. The precipitating antibody was not detectable across the IgG profile and did not correlate with the presence of high CF titers.

The results from testing the sera of 4 individuals against various antigenic strains of influenza A are shown in Table 2. The data suggests that the precipitating antibody developed by

individuals with influenza A<sub>2</sub> disease will also react with heterologous antigens of wide strain differences as well as homogenous or related influenza A<sub>2</sub> strains. Cross precipitation to influenza B antigen did not occur in sera giving a precipitin to influenza type A antigen.

Immunizations with inactivated bivalent influenza vaccine did not stimulate detectable levels of precipitating antibody, although the subjects did produce high (1:32 or greater) CF antibody levels to both influenza A and B antigens.

#### DISCUSSIONS

The CIE precipitating antibody phenomenon appears a specific immune response to active influenza infection with or without symptomatic disease. It is a broadly reacting antibody to both related and distant strains of influenza A but not to influenza type B.

Peculiarly as previously reported (1) it was not demonstrable following vaccination with inactivated influenza vaccine containing homologous serotypes and appears to require some component of the active infection process in order to be detectable. This aspect may be due to low sensitivity resulting from minimal antigenic stimulation as compared with an active infectious process. Further studies on animal hyperimmunization may elucidate this aspect of the CIE precipitin response.

It appears that the CIE antibody, like CF response, is contained exclusively in the IgG fraction of immune globulins, and thus would be expected to appear later. However, the two antibody types (CF and CIE) are not temporally or quantitatively



identical but this may be due to difference in avidity or other antigen combining characteristics of the two antibody molecules. However, this aspect in combination with lack of vaccination response may be used to advantage as an epidemiological tool.

It has often been our experience that impending outbreaks of influenza in human seroepidemiologic studies (unpublished observations) are preceded by increases in antibody titer prior to awareness of excess disease morbidity. Interpretation of these observations have been obscured by being unable to distinguish between natural and vaccine antibody responses or not having available the appropriate variant antigen. If the CIE technique continues to demonstrate its universal reactivity within an influenza serotype it will indeed prove to be a valuable asset in influenza epidemiological surveillance. The test is rapid, easy to perform, and economical.

Further work is needed to confirm and refine these observations.

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Table 1. Complement Fixation titers ( $\log_2$ ) and CIE results from fractions of human sera eluted from a Sephadex G200 column.

Fraction	CF Titer	CIE Test	CF Titer	CIE Test	CF Titer	CIE Test
	Subject #1		Subject #2		Subject #3	
1	8	-	8	-	2	-
2	8	-	8	-	8	-
3	8	-	4	-	8	1
4	16	+	64	+	16	+
5	16	+	64	+	64	+
6	32	+	128	+	64	+
7	32	+	128	+	64	-
8	32	+	128	+	64	-
9	32	+	128	+	32	-
10	32	-	128	-	8	-
11	32	-	128	-	8	-
12	8	-	64	-	4	-
13	8	-	32	-	4	-
14	8	-	32	-	2	-
15	8	-	16	-	2	-
16	4	-	16	-	<2	NT
17	4	-	16	-	<2	NT
18	4	NT	8	-	<2	NT
19	4	NT	8	-	<2	NT

NT: Not Tested

Table 2. Precipitating Antibody to Various Influenza A Strains by  
Individuals in Different Stages of Influenza A2 Disease.

Patient	CF	(H <sub>1</sub> N <sub>1</sub> ) (PR8)	(H <sub>1</sub> N <sub>1</sub> ) (Ann Arbor)	(H <sub>2</sub> N <sub>2</sub> ) (J170)	(H <sub>3</sub> N <sub>2</sub> ) (Hong Kong)	(H <sub>3</sub> N <sub>2</sub> ) (England)	B/MASS
S - Acute*	1:8	-	-	-	-	-	-
21 day conval	1:128	+	+	+	+	+	-
32 day conval	1:128	+	+	+	+	+	-
D - Acute**	1:256	+	+	+	+	+	-
H - Conval	1:64	+	+	+	+	+	-
E - Conval	1:128	+	+	+	+	+	-

\*Blood taken within 24 hr of illness (malaise, minimal symptoms), however,  
still working.

\*\*Blood taken 96 hrs after illness onset.

Figure 2. Relationship between complement fixation titer and CIE precipitin reaction in fractions from a Sephadex G-200 column.

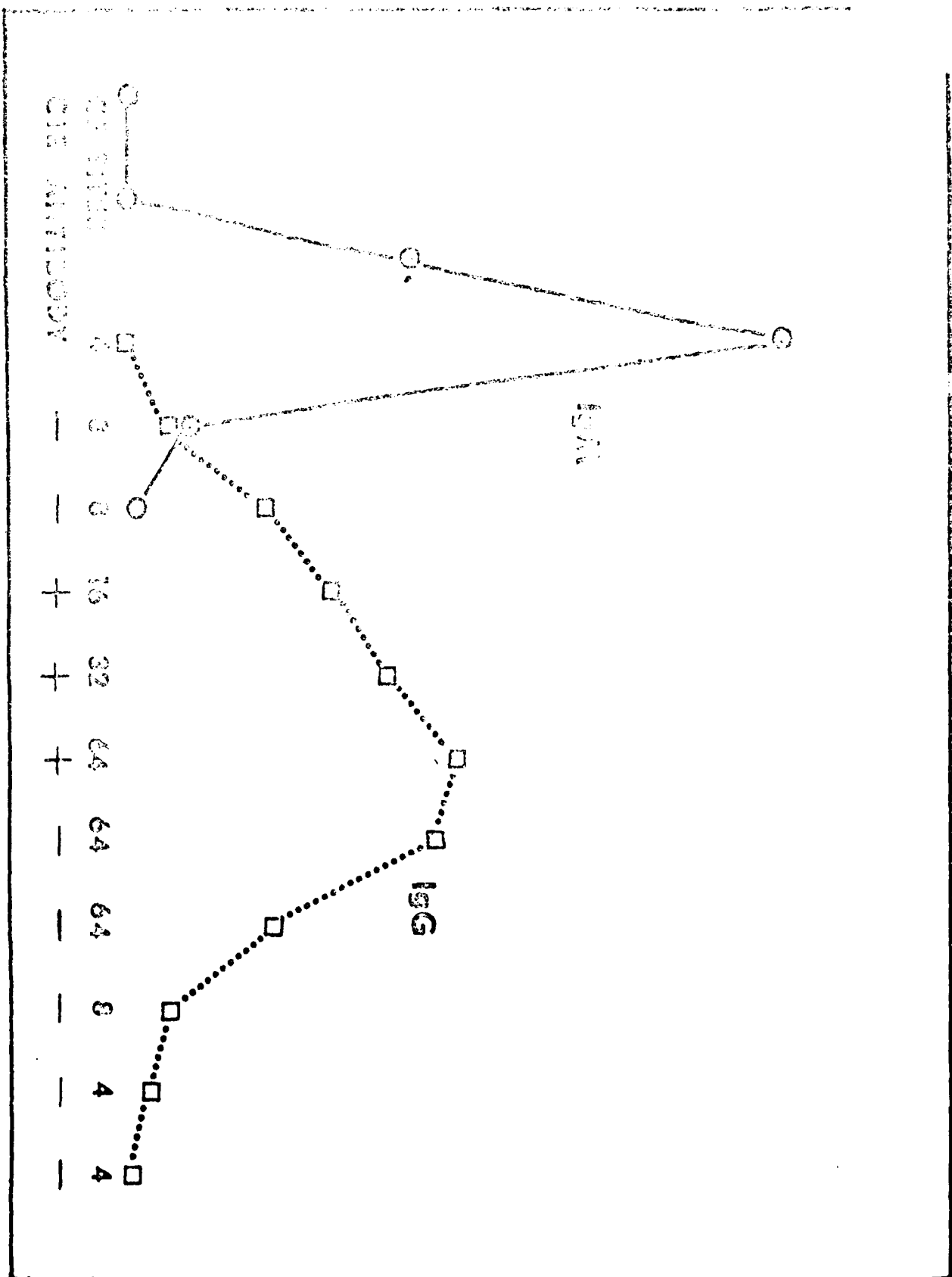
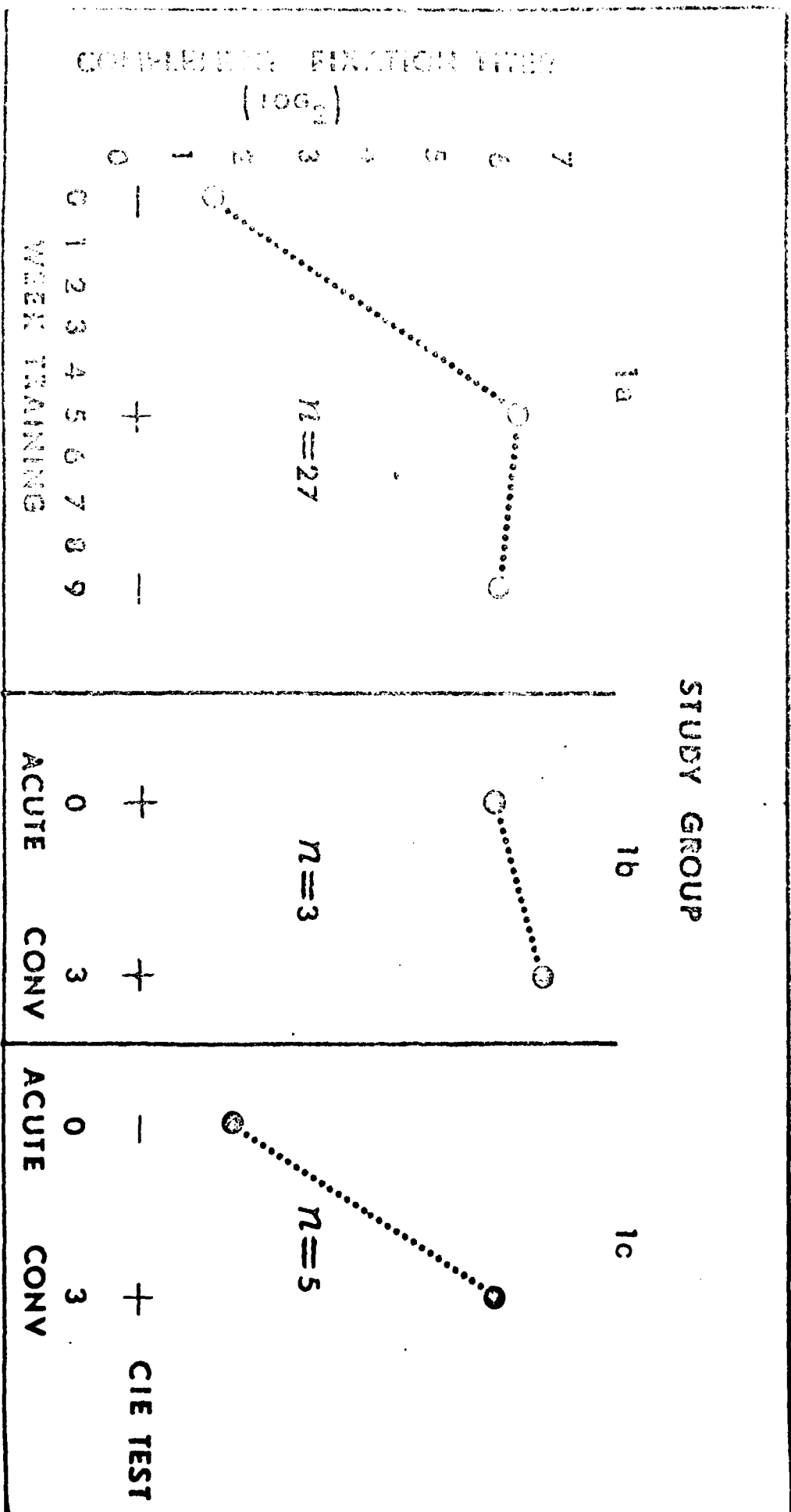


Figure 1. Complement fixation and CIE test results of 27 non-immunized men by week in recruit training. All 27 seroconverted to Influenza A. The CIE test was negative by the 9th week of training although the CF titers remained elevated (1a)., complement fixation and CIE test results when the CF titer was elevated at the same time of admission with Influenza A2 disease (1b)., and complement fixation and CIE test results when the CF titer was low in the acute phase of the disease (1c).



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